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What do Glands tell us about Species? A Chemical Case Study of *Cataglyphis* Ants

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Key Word Index—Insecta; Hymenoptera; Formicidae; *Cataglyphis altisquamis*; *Cataglyphis bicolor* group; *Cataglyphis cursor* group; *Cataglyphis frigidus*; *Cataglyphis isis*; *Cataglyphis mauritanicus*; *Cataglyphis niger*; *Cataglyphis nodus*; *Cataglyphis ruber*; Dufour gland; mandibular gland; chemotaxonomy.

Abstract—Chemical examination of 10 species of the genus *Cataglyphis* collected in Syria and Tunisia showed, in each case, that they can be readily distinguished from each other by the volatile contents of their exocrine glands. Five species of the *C. bicolor* group were chemically closely similar. The mandibular glands of all the species of the *C. bicolor* group contain 2-methyl-1-hexanol, a substance found as a natural product for the first time.

Introduction

Ants of the genus *Cataglyphis* Förster 1850 are distributed in the deserts, steppe and Mediterranean ecosystems of the Palearctic region, and, where present, always constitute a major component of the local insect fauna (Wehner *et al.*, 1983). The genus *Cataglyphis* includes 104 described (i.e. available species and subspecific names) and several undescribed species and subspecies (Agosti, 1990). Few of the species have a wide distribution, and most of the species are known only from their type locality or are endemic for very restricted areas of the Palearctic region, e.g. *C. fortis* (Wehner, 1983). At least the species of the *bicolor* and the *cursor* species group seem to form distinct taxa in a given geographical area, replacing each other ecologically (Agosti, 1990).

Most of the *Cataglyphis* species are scavengers (Wehner *et al.*, 1983; Wehner 1987). The main diet is dead insects which they collect individually. The size of their prey is variable, but if there are several *Cataglyphis* species in the same locality, there is a tendency for the larger species to collect the larger prey and the smaller species to collect smaller items.

From Syria four species groups of *Cataglyphis* are represented (*cursor, altisquamis, albicans* and *bicolor*; see Table 1). Of the seven species studied, four occur in the same or adjacent habitats, so that they do have casual interactions. The two very closely related *C. frigidus* and the *cursor* group species live on the Antilebanon mountains, *frigidus* on the tops of ridges, and the *cursor* group species on the adjacent north-facing slopes. *Cataglyphis ruber* and *C. niger* live in the foothills of the Antilebanon mountains, just beyond Damascus, and share the same locality and habitat. The remaining three species are from widely separated areas; *C. nodus* lives on the edge of the Oasis of Damascus, probably on drier sites than *C. niger, C. altisquamis* in the gardens of Suweida in the Djebel el Arab, and *C. isis* was collected in the salt lakes and the adjacent holophyte vegetation south of Palmyra.

The Tunisian species included here belong to the *altisquamis (mauritanicus)* and the *bicolor* groups [bright and dark red species of *bicolor, bicolor*-1 and *bicolor*-2 (Wehner,

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TABLE 1. LOCALITIES AND HABITATS OF THE *CATAGLYPHIS* SPECIES*

Group and species	Nest no.	Locality
altisquamis group		
altisquamis		Syria, Sursida
mauritanicus	B_1	Tunisia, Siliana, Bargou
	B_2	Tunisia, Siliana, El Fahs
	B_3	Tunisia, Soliman, Grombalia
cursor group		
frigidus		Syria, Antilebanon mts Blondan
cursor group sp.		Syria, Antilebanon mts Blondan
bicolor group		
isis		Syria, Palmyra, Saltlake
niger	009	Syria, Damascus
	041	Syria, Palmyra
	154	Syria, Damascus
nodus		Syria, Damascus, Diabieh
bicolor-1	A_1, A_2	Tunisia, Makhtar,
	A_3, A_4	Siliana,
	A_5, A_8	Sidi Rais,
	A_6, A_7	Kairouan
bicolor-2	C_1	Tunisia, Douz,
	C_2	Kairouan,
	C_3	Mahaies,
	C_4	El Fahs
Albicans group		
ruber		Syria, Damascus

*Voucher specimens are deposited in the collection of Agosti.

1987) respectively]. Ecologically, the dark red species (*bicolor*-2) is distributed in steppe type habitat and the *mauritanicus* and the bright red species (*bicolor*-1) more in the northern Mediterranean type habitats. The dark and bright red species have a narrow zone of overlap in their distribution; they are of about the same size as the *mauritanicus*, which often co-occurs with them (Wehner and Wehner, in preparation).

At higher taxa levels in *Cataglyphis*, morphological variation is very distinct and discrete and can be used for phylogenetic studies. The problems lie at the species level, because morphological characters are lacking, neither can the species be diagnosed sufficiently nor can the phylogenetic relationships of the species be easily inferred (Agosti, 1990). This greatly hinders evolutionary biological interpretation of current chemical (Hefetz and Orion, 1982; Hefetz and Lloyd, 1985; Ali *et al.*, 1988; Attygalle and Morgan, 1984) physiological (Wehner, 1989; Wehner and Lanfranconi, 1981; Wehner and Muller, 1985; Lighton and Wehner, 1990) and eco-ethological studies (Santschi, 1929; Wehner, 1987; Nowbahari *et al.*, 1990; Wehner *et al.*, 1983).

All ants are eusocial, i.e. exhibit brood care, generation overlap and a worker or non-reproductive caste (Wilson, 1971). Communication within the colonies is largely based on chemical secretions, which is correlated with an extremely high number of glands in ants in comparison with other insect groups (Hölldobler and Wilson, 1990). From studies of the exocrine secretions of many ant species made over recent years, it is apparent that these secretions vary in composition from species to species. Hefetz and Orion (1982) have examined the volatiles of the Dufour glands of six species of *Cataglyphis* collected in Israel (*C. niger, C. nodus, C. viaticoides, C. lividus, C. isis* and *C. bombycinus*), and Hefetz and Lloyd (1985) have examined the mandibular gland secretion of *C. bombycinus* and *C. niger.* We have reported a study of *C. savignyi* from Egypt (Ali *et al.*, 1988). On the other hand, Nowbahari *et al.* (1990) have used the cuticular hydrocarbons of *C. cursor* as a taxonomic marker and concluded from the three areas in France and Spain that subspecies could be identified there, and correlated chemical differences with behavioural tests of aggressiveness between the groups.

While cuticular hydrocarbons may have an active role in kin, colony and species recognition (Bagnères and Morgan, 1991), chemically they are a complex mixture of very similar hydrocarbons, so that any analysis of differences requires detailed statistical treatment. The exocrine gland secretions show a greater variety of compounds and fewer components. There were sufficient differences apparent in those examples already studied to encourage us to look more closely at some examples all taken from an area where they co-occur.

The aim of this study was to take a further step towards the species level analysis in the taxonomy of *Cataglyphis*, by using the diagnostic power of the chemical compounds of the antennal, Dufour, mandibular, metapleural and postpharyngeal glands of ants from four species groups of *Cataglyphis* (*cursor, altisquamis, albicans* and *bicolor*) from southern Syria where they co-occur, and from Tunisia (Table 1).

In this study, the chemical compounds are described, with their distribution and composition of mixtures among the different species, and their diagnostic power at species level is elucidated. The use of the chemical compounds in a phylogenetic analysis will be dealt with in a subsequent paper (Agosti *et al.*, in preparation). Both are part of a revisionary study of the genus *Cataglyphis* (Agosti, 1990; Agosti *et al.*, in preparation).

Materials and Methods

Collection and preparation of insect material. Three separate collections of glands were made for chemical examination. All individuals were foraging workers captured near their nests (Table 1). They were dissected the same day and Dufour glands and mandibular glands (either paired or singly) were sealed in soft glass capillaries, and packed in labelled containers for transport home. In May 1988, in addition to Dufour and mandibular glands, some metapleural glands, antennal glands and postpharyngeal glands were also collected, and similarly sealed in glass capillaries (Morgan, 1990).

In May 1989 more samples of each of seven species were collected in the same area, and, as far as possible, from the same nest. Mandibular and Dufour glands only were collected, similarly sealed in glass capillaries. The samples from Tunisia in May 1989 and June 1989 were collected and prepared similarly, but species names were not given and only a code of nest sites was used until after all the results of chemical tests were presented. Voucher specimens are in the collection of D. Agosti, Zürich.

Chemical analysis. Linked gas chromatography–mass spectrometry was carried out directly on the sealed samples of glands by the solventless solid injection method of Morgan and Wadhams (1972), using a Hewlett Packard Model 5890 gas chromatograph linked to a Model 5970B Mass Selective Detector, controlled by an HP 59970C ChemStation. A fused silica capillary column (12 m×0.2 mm i.d.) coated with a 0.33 μm film of immobilized polydimethylsiloxane (equivalent to OV-1) was used. Helium at 10 p.s.i. head pressure (1 ml min^{-1}) was used as carrier gas.

A sealed capillary containing a gland was placed in the injection area, which was heated to 150°C for mandibular and Dufour glands. After 2 min, the gas capillary was crushed and the chromatogram commenced. A common temperature programme was used for all the glands. The initial oven temperature of 30°C was increased at 8°C min^{-1} to 150°C for mandibular glands and 250°C for Dufour glands. A higher temperature programme was used for the postpharyngeal glands. The injector was set at 250°C and the oven was programmed from 150°C at 3°C min^{-1} to 250°C. The mass spectrometer was set to scan *m/z* 25–150 for mandibular glands and *m/z* 35–350 for all other systems.

Quantification. The amounts of the substances found in the glands were calculated by comparing peak areas by electronic integration of the natural substances with those of known amounts of standard substances of similar structure and in similar quantities. Pentadecane was used as a standard for the Dufour gland substances and citronellol was used as the mandibular gland standard.

Identification. Substances were identified from their mass spectra and except for alkanes and alkenes, were confirmed by comparison of retention time and spectra with those of a synthetic specimen. Commercial samples of nonanol, decanol, dodecanol, geraniol, citronellol and citronellal were available. A mixture of farnesene isomers, produced by dehydration of nerolidol was available in the laboratory. Dodecyl acetate was prepared on a small scale.

2-Methyl-1-hexanol was prepared via a Grignard reaction on 2-bromohexane (43 mmol) and formaldehyde (53% yield). The structure of the product 2-methyl-1-hexanol was confirmed by spectroscopy [IR, 3360 cm^{-1} (OH), 2926 (C—H) 1460, 1160, 1034 cm^{-1}]. NMR at 270 MHz δ 0.9 (*d*+*t*, 6H, Me—CH$_2$ and MeCH), δ 1.4 (m 3CH$_2$), δ 1.7 (*m* CH), δ 1.9 (1H OH), δ 3.40 (*d,d, J* 5.86, 10.5 Hz, CH—OH), δ 3.50 (*d,d J* 5.86, 10.5 Hz, CH—OH). The retention time and mass spectrum were identical to that of the natural material from the mandibular glands (see Fig. 1).

Results

Dufour glands

The first specimens were collected in the Syrian desert in May 1988. From their chemical analysis they appeared to show species differences, but the samples were too small for the results to be reliable. A further set of larger samples from seven species was collected from the same site in May 1989, so that quantitative measures of composition could be made. In a further test, samples from three species were collected in Tunisia in May and October 1989 and subjected to the same kind of analysis, but in this case the species were not disclosed to those involved in the chemical analysis.

In the initial collection, four specimens of workers of *C. niger* were taken from one nest and three other individuals from two different nests to see if there was consistency of composition of the glandular secretion within a colony and from one colony to another, at least in the same district. The results of this examination are shown in Table 2. Some 33 compounds are listed there as present in some or all of the glands. These compounds are chiefly linear alkanes and alkenes, dominated by tridecane and

TABLE 2. PERCENTAGE COMPOSITION OF DUFOUR GLAND SECRETIONS OF FORAGING WORKERS OF *C. NIGER*

	nest 041						nest 154		nest		
Compound	1	2	3	4	$\bar{x}_1$	±S.D.	1	2	009	$\bar{x}$	±S.D.
Undecene	t		t		t					t	
Undecane	4.6	2.9	3.5	6.3	4.3	1.3	—	9.7	3.9	4.4	2.8
3-Methylundecane	0.1	0.1			t			0.1		0.1	
Dodecene	0.1		0.2	1.1		0.4	0.4			0.2	0.4
Dodecane	0.4	0.3	0.5		0.3	0.2	0.2	1.2	0.7	0.5	0.4
Tridecene*	0.1		0.3	0.3	0.2	0.1		0.1	0.2	0.1	0.1
Tridecene*			0.1		t			0.2	0.1		
Tridecane	**22.7**	**14.6**	**15.0**	**18.1**	**17.6**	3.2	**11.9**	**23.5**	**15.4**	**17.3**	4.0
5-Methyltridecane							0.1	0.1		—	
3-Methyltridecane								0.1		—	
Tetradecane	3.9	5.4	4.6	6.2	5.0	0.9	5.5	5.8	6.7	5.4	0.8
1-Dodecanol	0.7	0.9	2.8	1.5	1.5	0.8	0.7	0.3	1.6	1.2	0.8
Pentadecadiene			0.1		t		0.2		0.3	—	
Pentadecene*			0.3	0.4	0.2	0.2	0.7	0.6	0.6	0.7	0.5
Pentadecene*			0.8	0.7	0.4	0.3	0.2		0.3	—	
Pentadecane	**45.1**	**51.9**	**47.4**	**46.8**	**47.8**	2.5	**71.3**	**46.5**	**53.6**	**51.8**	8.4
5-methylpentadecane			0.1	0.3	0.2	0.1	0.1	0.1	0.1	0.1	
3-Methylpentadecane	0.2	0.3	0.1	0.2	0.2	0.1	0.1	0.1	0.1	0.1	
Dodecyl acetate	0.4	0.3	0.2		0.2	0.2			0.6	0.2	0.2
Hexadecane	0.2		0.4	0.5	0.3	0.1	0.4	0.3	0.6	0.3	0.2
1-Tetradecanol		0.3	0.4		0.2					0.1	0.1
Heptadecadiene	0.1				t				0.2	t	—
2-Pentadecanone			5.4	4.9	2.6	2.5	3.2	2.7	3.6	2.8	2.0
2-Pentadecanol	3.0	2.6	0.5	0.5	1.7	1.1	0.4	0.2	0.3	1.1	1.0
Heptadecane	0.4	0.3	7.1	8.3	4.0	3.6	3.0	3.5	8.2	4.4	3.2
1-Hexadecanol	4.5	3.8			2.1	2.5				1.2	1.9
Octadecene			0.4		t		t	t	0.1	t	
Octadecane	0.1	0.3	0.1	0.3	0.2	0.1	0.1	0.4	0.3	0.2	0.1
2-Heptadecanone	1.9	2.6	6.9	3.0	3.6	1.9	0.7	2.6	1.7	2.9	1.7
2-Heptadecanol			0.1		t		0.3	0.2		t	
Nonadecane	2.3	3.2	3.3	1.6	2.6	0.7	1.2	2.4	1.8	2.2	0.7

These nests labelled 041, 154 and 009, were sampled with four individuals from the first: two from the second and one from the third. $\bar{x}_1$ values are the means from nest 041; $\bar{x}$ are the mean values for all individuals. Compounds are listed in order of elution from the chromatography column. The principal substances are shown in bold. t = trace amount.

*Isomers separated by chromatography.

pentadecane but also including smaller amounts of alcohols, alkanones, methyl-branched alkanes and one acetate. The individual values for each of seven workers, together with the mean values, n_1 and the sample standard deviation for the four samples from nest 041 are given, and the mean *n* and standard deviation of all the samples. Inspection shows that there is variation from one individual to another but within the single nest or from one nest to another; the composition is constant. Using the independent *t*-test, there was no statistically significant difference in the content of tridecane and pentadecane in nests 041 and 154 at $P = 0.10$.

Hydrocarbons with an odd number of carbon atoms make up about 80% of the total contents. Two tridecene and two pentadecene isomers with different retention times were separated in chromatography but the double bond positions have not been identified in these minor components. The limit of detection of these substances is between 10^{-8} and 10^{-9} g per individual insect. One or two samples from seven other species were also analysed but the results are not recorded here.

Ten specimens of the same species and of six others were then collected, so that better mean and standard deviations could be provided to represent the composition of each species. Each individual worker was analysed in the same way. The mean values for the composition of the Dufour gland secretion are shown in Table 3. The ANOVA test was used to look at differences in content of tridecane, pentadecane, dodecanol and dodecyl acetate between the seven species. In every case, the differences were highly significant at the $P = 0.001$ level.

A total of 14 nests of three species from Tunisia were sampled in the same way (Table 1). The two species of the *bicolor* group are known only as *C. bicolor*-1 and *C. bicolor*-2; the third, *C. mauritanicus*, belongs to the *altisquamis* group. The largest number of specimens were collected from *C. bicolor*-1, for which the mean values for each nest and for all the samples are summarized in Table 4. Similarly the results for *C. bicolor*-2 are given in Table 5. Although there is more variation between individuals than we found in the Syrian samples (compare the sample standard deviations in Tables 2, 4 and 5), these species are chemically close to *C. niger* and *C. nodus*, two other species from the *bicolor* group, than they are to any of the other species. There were only seven specimens of *C. mauritanicus* (Table 6). A further nest, sent as belonging to this species, on chemical analysis was found not to fit with *C. mauritanicus*, and on checking was found wrongly labelled, and was indeed samples of *C. bicolor*-2. The mean values of composition of the glands of these species have been added to Table 3.

Mandibular glands

The first set of samples were variable. Some samples contained no volatile substances in the glands and the composition in those that did have volatiles seemed to vary within the species.

When 10 samples from each species were collected, much more reliable results were obtained (Table 7). The absence of volatiles seems to be a problem of preparation of the sample for in some cases the two mandibular glands were carefully dissected out of the head and each sealed in a separate capillary. Sometimes one gland contained much more material than the other, or one or both were empty. There was no evidence of a change of composition with amount in the gland.

The results for *C. bicolor*-1 mandibular glands (Table 8) showed more than usual variation. The single samples from nests A3 and A4, showed a different composition from the others. The results for *C. bicolor*-2 (Table 9) were self-consistent, as were those for the three nests of *C. mauritanicus* (Table 10).

Citronellol was the major substance present in *C. altisquamis* and *C. frigidus*; the *C. cursor*-group species contained a mixture of citronellol and farnesene (Table 7). The four species of the *bicolor* group (Table 1) contained a primary C_7 alcohol, which by

TABLE 3. THE PERCENTAGE COMPOSITION OF THE CONTENTS OF THE DUFOUR GLAND OF SEVEN SPECIES OF *CATAGLYPHIS* ANTS FROM SYRIA AND THREE FROM TUNISIA

				bicolor group						
Compound	*C. altisquamis*	*C. cursor* group	*C. frigidus*	*C. isis*	*C. niger*	*C. nodus*	*C. ruber*	*C. bicolor*-1	*C. bicolor*-2	*C. mauritanicus*
Undecene	0.4	0.1	t	t	t	t	t	t		
Undecane	11.8	3.1	**20.2**	3.2	5.2	2.4	1.2	3.6	8.7	**21.7**
5-Methylundecane		0.1	t					t		
3-Methylundecane		0.1	t	t	0.1		t	t	0.3	t
Dodecene								0.2	t	0.2
Dodecane	0.1	0.3	0.8	0.4	0.5	0.3	0.1	1.4	1.3	3.3
1-Decanol	0.1		t							
Tridecene	0.4	0.5	0.3	0.2	0.3	0.1	0.1	t	0.3	1.0
Tridecane	**18.6**	**16.7**	**44.6**	**53.6**	**19.1**	**26.7**	**15.8**	**39.7**	**26.6**	**63.9**
5-Methyltridecane		0.1	0.2	0.2	0.1	0.1		0.6	0.3	
3-Methyltridecane		0.3	0.2		0.1	0.1	0.3	5.0	1.4	0.1
Decyl acetate	0.8	0.2		t			t	t		
Tetradecene								1.0	t	
Tetradecane	0.6	0.4	0.4	0.2	5.5	2.4	1.4	2.4	3.8	0.1
Undecyl formate			t							1.8
1-Dodecanol	**10.6**	**23.5**	**10.9**	**40.0**	1.3	0.3	**15.8**	3.5	0.4	1.2
Pentadecene	0.7	0.6	0.5	0.3	1.1	0.6	0.8	0.4.	0.5	0.4
Pentadecane	9.7	7.8	13.6	9.1	**48.6**	**42.9**	**53.9**	**30.2**	**48.9**	5.0
1-Tridecanol		0.1		t	0.5	0.4	0.3			
5-Methylpentadecane		0.4	0.2	t		t	0.6	1.1	2.7	
3-Methylpentadecane		0.3	0.5		0.3	0.3	0.2	1.7	0.6	
Dodecyl acetate	**41.2**	**39.5**		7.1	0.2	0.2	4.0	4.1		
Tridecyl acetate	0.1	0.2		t	t	0.1	0.3	1.5		
Hexadecane			0.3	0.2	0.2	0.4	0.2	0.8	2.4	0.1
Tetradecyl acetate	1.2	1.2		0.9			0.9	t		
2-Pentadecanone					3.7	6.5	t	t	0.3	
2-Pentadecanol					0.4	0.9	0.6		0.1	
Heptadecene	0.3	0.3	0.7	0.5	0.2	0.3	t			
Heptadecane	0.2	2.0	2.9	0.8	5.9	8.4	0.4	0.7	t	0.6
Dodecyl propionate		0.7								
Tetradecyl propionate		0.2	t	0.1			0.1			
Octadecene		t	t	0.5	0.2	0.1	0.2			
Octadecane		0.1	0.3		0.2	0.3	0.2		0.4	
2-Heptadecanone	0.2	0.2	0.1		3.1	1.9			0.4	
2-Heptadecanol	0.5		0.2	0.2	0.4					
Nonadecene		0.3		1.1	0.3	0.4	0.7			
Nonadecane		1.-2		0.8	2.4	0.4	t		0.4	0.7
Total amount (μg)	3.5	1.2	0.8	0.7	1.6	1.6	1.0			

Each number represents the mean from 10 individual workers for the Syrian samples. The mean values from the Tunisian species, taken from Tables 4–6 are shown for comparison. The principal substances are shown in bold. t = trace amount.

TABLE 4. PERCENTAGE COMPOSITION OF THE DUFOUR GLAND CONTENTS OF INDIVIDUALS FROM EIGHT NEST SITES OF *C. BICOLOR*-1 FROM TUNISIA

	Nest									
	A1	A2	A3	A4	A5	A6	A7	A8	$\bar{x}$	±S.D.
	No. of individuals									
Compound	6	1	1	1	5	4	5	4	27	
Undecene	t	t					0.2	t	t	—
Undecane	3.8	2.6	2.5	3.1	4.3	4.3	5.3	3.0	3.6	1.0
5-Methylundecane	0.1				t	t	0.1	0.1	t	—
3-Methylundecane	0.1				0.6	t	0.3	0.7	t	—
Dodecene	t	0.4		0.4	0.3	0.3	t	t	0.2	
Dodecane	1.4	0.9	1.1	1.2	2.6	1.6	1.2	1.1	1.4	0.5
Tridecene	t				t	0.3	0.3	0.2	t	
Tridecane	**41.0**	**50.9**	**42.3**	**40.2**	**18.3**	**37.7**	**59.8**	**27.9**	**39.7**	12.7
5-Methyltridecane	0.7	0.4		1.2	0.7	0.2	1.3	0.3	0.6	0.4
3-Methyltridecane	5.4	3.0	6.6	5.4	5.3	0.3	4.6	3.5	5.0	1.3
Decyl acetate	0.8								t	
Tetradecene	0.1	1.2	2.5	3.2	0.4	0.2	0.2	0.2	1.0	0.2
Tetradecane	2.2				6.4	2.3	3.5	4.6	2.4	2.4
1-Dodecanol	0.7	4.3	3.1	2.8	13.6	2.7		0.5	3.5	4.4
Pentadecene	0.3	0.2	0.3	0.6	0.3	0.4	0.3	0.4	0.4	0.1
Pentadecane	**22.2**	**18.7**	**37.1**	**20.9**	**30.4**	**37.8**	**25.0**	**49.4**	**30.2**	10.6
5-Methylpentadecane	0.9	0.2	0.9	0.4	2.1	1.7	0.7	2.0	1.1	0.7
3-Methylpentadecane	0.8	0.6	2.5	1.0	5.5	1.3	0.3	1.7	1.7	1.7
Dodecyl acetate	13.6	12.3	0.2	4.8		0.9		1.0	4.1	5.7
Tridecyl acetate	4.1	3.2	0.1	3.7	0.2	0.3			1.5	1.8
Hexadecane	0.4	0.4	1.3	0.9	1.6	0.7	0.4	0.5	0.8	0.5
Tetradecyl acetate	0.5					t			t	
Pentadecyl acetate	0.3								t	—
2-Pentadecanone	0.2					t		0.1	t	
Heptadecane	0.7	0.5	1.1	1.0	1.6	0.3		0.6	0.7	0.5

The principal substances are shown in bold.

t = trace amount; $\bar{x}$ = mean of all samples together with its standard deviation.

comparison of its mass spectrum (Fig. 1) and retention time with synthetic samples of several candidates, was shown to be 2-methyl-1-hexanol, a substance not previously found as a natural product. The glands of *C. isis* and *C. ruber* were mostly empty, four samples of *C. isis* contained methylhexanol as the major compound and three samples of *C. ruber* contained small amounts of nonanal and decanal (cf. Table 7). The mean values of composition for the Tunisian species have been added to Table 7 for comparison.

Metapleural and antennal glands

A number of samples of metapleural glands were collected in the first visit to Syria. Half of these contained no detectable volatile material. The others contained variable traces of substances, such as hydrocarbons (chiefly C_{15}) perhaps derived from the Dufour glands, or traces of nonanal and decanal. It was concluded that the metapleural glands did not contain sufficient amounts of volatile substances and no further glands were collected. Samples of antennal glands also showed no evidence of volatile contents.

Postpharyngeal glands

We have recently shown that the postpharyngeal glands of several species of ant contain complex mixtures of C_{20} to C_{30} hydrocarbons, very close in composition to the hydrocarbons on the cuticle (Bagnères and Morgan, 1991). All the postpharyngeal glands collected in the first visit to Syria contained complex mixtures of higher hydrocarbons. The composition differed with species, e.g. *C. altisquamis* had 3-methyl-

TABLE 5. MEAN PERCENTAGE COMPOSITION (where there is more than one specimen) OF THE DUFOUR GLAND CONTENTS OF INDIVIDUALS FROM FIVE NEST SITES OF *C. BICOLOR*-2 FROM TUNISIA, AND THE MEAN SAMPLE STANDARD DEVIATION FOR THE WHOLE GROUP

	Nest					
	C_1	C_2	C_3	C_4	$\bar{x}$	S.D.
	No. of individuals					
Compound	4	1	3	3	11	
Undecane	9.8	8.3	5.4	9.9	8.7	3.5
3-Methylundecane	0.6	0.6	0.2	0.1	0.3	0.4
Dodecene	t	—	t	0.2	t	—
Dodecane	1.3	0.8	2.4	1.8	1.3	1.1
Tridecene	0.2	0.3	0.4	0.2	0.3	0.1
Tridecane	**26.4**	**24.8**	**26.8**	**27.2**	**26.6**	4.9
5-Methyltridecane	0.3	—	0.2	t	0.3	0.6
3-Methyltridecane	1.3	1.4	1.7	1.5	1.4	1.1
Tetradecene	—	—	0.2	t	t	—
Tetradecane	3.2	2.9	5.2	4.0	3.8	1.7
1-Dodecanol	1.0	—	0.7	0.4	0.4	0.7
Pentadecene	0.2	0.2	0.8	0.5	0.5	0.2
Pentadecane	**46.9**	**56.3**	**46.9**	**52.0**	**48.9**	11.9
7-Methylpentadecane	0.2	—	—	—	t	—
5-Methylpentadecane	0.5	0.4	6.2	1.0	2.7	3.5
3-Methylpentadecane	0.8	1.8	1.1	0.3	0.6	0.8
Hexadecane	0.6	0.3	1.2	0.6	2.4	2.4
2-Pentadecanone	0.2	—	—	—	0.3	0.9
2-Pentadecanol	1.5	1.1	—	—	0.1	0.4
Heptadecane	3.3	—	—	—	t	—
Octadecane	0.4	—	t	t	0.4	0.5
Nonadecane	0.5	—	—	—	0.4	0.9
2-Heptadecanone	0.8	0.6	0.6	0.2	0.4	0.3

t = trace amount.

TABLE 6. MEAN PERCENTAGE COMPOSITION OF THE DUFOUR GLAND CONTENTS FOR EACH NEST AND FOR THE WHOLE SAMPLE FROM THREE NESTS OF *C. MAURITANICUS* FROM TUNISIA, TOGETHER WITH THE SAMPLE STANDARD DEVIATION

	Nest				
	B_1	B_2	B_3	$\bar{x}$	S.D.
	No. of individuals				
Compound	1	5	1	7	
Undecane	**19.0**	**23.3**	**22.3**	**21.7**	2.3
3-Methylundecane	0.6	t	—	t	—
Dodecene	0.1	0.2	—	0.2	0.1
Dodecane	1.6	3.0	5.4	3.3	1.6
5-Methyldodecane	—	t	—	t	—
3-Methyldodecane	0.2	0.2	—	0.2	—
Tridecene	0.5	0.6	1.8	1.0	0.6
Tridecane	**69.4**	**63.6**	**58.8**	**63.9**	4.3
3-Methyltridecane	—	0.2	—	0.1	—
Undecyl formale	2.3	2.7	—	1.8	1.8
Tetradecene	0.1	0.1	—	0.1	0.2
1-Dodecanol	—	0.1	3-5	1.2	1.6
Pentadecene	0.2	0.4	—	0.4	0.2
Pentadecane	5.4	4.8	4.7	5.0	0.3
5-Methylpentadecane	—	0.1	—	—	—
3-Methylpentadecane	—	0.1	t	—	—
Hexadecane	—	0.1	0.2	0.1	—
Heptadecadiene	—	0.2	—	—	—
Heptadecane	0.6	0.4	0.8	0.6	0.2
Nonadecane	—	—	2.0	0.7	0.9

t = trace amount.

TABLE 7. THE PERCENTAGE ABUNDANCE OF THE COMPOUNDS FOUND IN THE MANDIBULAR GLANDS OF SEVEN SPECIES OF *CATAGLYPHIS* FROM SYRIA AND THREE FROM TUNISIA

		cursor group		*bicolor* group						
Compound	*C. altisquamis*	*C. cursor*	*C. frigidus*	*C. isis**	*C. niger*	*C. nodus*	*C. ruber*†	*C. bicolor*-1	*C. bicolor*-2	*C. mauritanicus*
2-Methyl-1-hexanol		1.4		**53.4**	**96.5**	**91.8**		**83.7**	**99.2**	
Octanal				0.4		0.3		0.7		
Nonanal	0.9	2.2	0.7	18.3	1.2	11.3	48.2	0.6	0.2	
Citronellal	4.2	4.9	1.3	1.9		1.0		t		4.9
Decanal		4.2	0.5	21.2	1.8	7.2	52.6	2.4	0.4	3.4
1-Decanol	t									
Citronellol	**96.5**	**51.2**	**96.4**			1.3		8.3	0.8	**89.8**
(*Z,E*)-α-Farnesene	2.3	40.4								
(*E,E*)-α-Farnesene	1.6	2.9								
Geraniol								2.-6	t	2.9
Citronellyl acetate	t	0.7								
(*E,E*)-Farnesol		4.2								
Dodecyl formate								t		
1-Dodecanol								0.9		
1-Tetradecanol								0.2		
Total amount (ng)	996	63	241	8	45	51	9			

Results are the means of 10 specimens from Syria. The mean values for the Tunisian species are taken from Tables 8–10 for comparison.

*Six out of the 10 samples contained very little volatile material.

†Seven out of the 10 samples contained no volatiles.

t = trace amount.

TABLE 8. PERCENTAGE COMPOSITION OF THE SECRETION OF THE MANDIBULAR GLAND OF WORKERS FROM EIGHT DIFFERENT NESTS OF *CATAGLYPHIS BICOLOR*-1

	Nest A1	A2	A3	A4	A5	A6	A7	A8	$\bar{x}$	S.D.
	No. of individuals									
Compound	6	1	1	1	5	4	5	4	27	
2-Methyl-1-hexanol	**93.6**	**96.6**	**50.6**	**77.4**	**96.8**	**90.4**	**78.1**	**98.9**	**83.7**	16.8
Octanal		2.3	2.4		0.9				0.7	0.9
Nonanal	0.2	1.4	2.4		0.8	0.7			0.6	0.8
Citronellal						0.1			t	
Decanal	1.2				0.5	0.2	11.6		2.4	4.6
Citronellol	4.3		**39.7**	9.3	0.7	6.3	8.4	0.3	8.3	12.0
Geraniol	0.7		5.0	12.7			2.0	0.8	2.6	4.4
Dodecyl formate						0.2				
1-Dodecanol				1.1					0.9	2.0
1-Tetradecanol					0.3		0.6		0.2	0.4

The mean for each nest and the mean with sample standard deviation of all the samples are given.

TABLE 9. PERCENTAGE COMPOSITION OF THE SECRETION OF THE MANDIBULAR GLANDS OF WORKERS OF *C. BICOLOR*-2, SHOWING MEAN VALUES FOR EACH NEST AND THE MEAN WITH STANDARD DEVIATION FOR THE WHOLE SAMPLE

	Nest C_1	C_2	C_3	C_4	$\bar{x}$	S.D.
	No. of samples					
Compound	4	1	3	3	11	
2-Methyl-1-hexanol	**99.2**	**100**	**94.4**	**100**	**99.2**	1.4
Nonanal	0.8	—	0.8	—	0.2	1.0
Decanal	—	—	1.5	—	0.4	1.0
Citronellol		—	3.0	—	0.8	2.5
Geraniol	—	—	0.3	—	t	—

TABLE 10. PERCENTAGE COMPOSITION OF THE SECRETION OF THE MANDIBULAR GLANDS OF WORKERS OF *C. MAURITANICUS* SHOWING MEAN VALUES FOR EACH NEST AND THE MEAN WITH STANDARD DEVIATION FOR THE WHOLE SAMPLE

	Nest B_1	B_2	B_3	$\bar{x}$	S.D.
	No. of individuals				
Compound	1	5	2	8	
Citronellal	—	—	12.2	4.9	11.1
Citronellol	**90.0**	**92.2**	**86.8**	**89.8**	10.3
Decanal	1.6	4.7	0.4	3.4	4.9
Geraniol	8.4	3.1	0.5	2.9	2.8

heptacosane as the largest component, *C. nodus* had pentacosane and the *C. niger* samples had an unresolved mixture of 11-methyl- and 13-methylnonacosane. Because of the relative complexity of these mixtures, they were considered at the time to be unpromising material to use for diagnosis of species compared with the Dufour and mandibular glands, so they were not collected in the further samples.

Discussion

The primary aim of this study was to explore the role of chemical compounds in exocrine secretions as taxonomic characters to separate the species *Cataglyphis*. The initial study was helpful in that it showed for one species that composition of Dufour

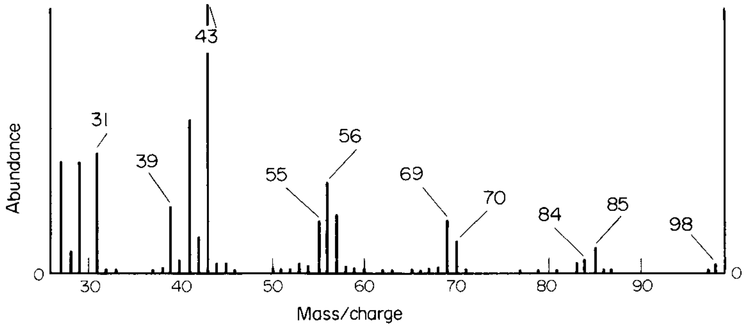

FIG. 1. MASS SPECTRUM OF NATURAL 2-METHYL-1-HEXANOL FROM *CATAGLYPHIS NIGER.*

and mandibular gland contents was relatively constant, but other glands collected in this series were less useful. Metapleural glands are found in most ants. In a few cases, volatile substances, notably carboxylic acids, have been found in this gland (reviewed in Attygalle and Morgan, 1984). No volatile substances were detected in our preliminary examination, but the type of column used meant that nanogram quantities of carboxylic acids would not be detected. Although the hydrocarbons of the post-pharyngeal gland turned out later to be a useful distinguishing feature between *C. nodus* and *C. niger* this was not recognized at the time of collection. Further sampling was therefore confined to mandibular and Dufour glands.

The number of substances detected depends upon the limiting sensitivity of the method, which is less than 10 ng as used here. Substances detected near that limit and described as traces in the table are of limited use in diagnosing species. In larger glands, small quantities may be seen, while in smaller glands the quantity might be just below the level of detection and would then be recorded as absent. The problem is analogous to using features just discernible at the magnification limit of a microscope in a biological context.

Dufour glands

In general, the substances are typical of formicine Dufour glands, with chiefly linear alkanes, some methylbranched alkanes and some oxygenated compounds (esters, alcohols and ketones) (cf. Ali *et al.*, 1988). Formicine ants from temperate climates usually have undecane as the major component, and as one moves to hotter climates, the major component moves to tridecane and pentadecane; *C. savigny* from Egypt had a similar, but recognizably different composition from those examined here (Ali *et al.*, 1988). The shift to less volatile major components is less evident here.

It is seen first from Table 2 that the four individual foragers from a single nest of *C. niger* have approximately the same composition of substances in their Dufour glands, and that when samples from two other nests of the same species are included the mean composition and sample standard deviations are essentially unaltered. It was concluded that within a range of values indicated by the standard deviations, the composition for the species was constant.

With larger samples of seven species, clear species differences were evident in all but two cases. *Cataglyphis niger* and *C. nodus* are very similar, although in the examples analysed, *C. niger* consistently had about equal quantities of 2-pentadecanone and 2-heptadecanone, while *C. nodus* always had more 2-pentadecanone than 2-heptadecanone. This chemical similarity parallels a morphological similarity for these species. *Cataglyphis altisquamis* and the *C. cursor* group species are both notable for the large amount of dodecyl acetate (the largest component in both), though they differ in the amount of dodecanol. *Cataglyphis frigidus* is distinguished by large amounts of tridecane, undecane, pentadecane and dodecanol (decreasing in that order). *Cataglyphis isis* has large amounts of tridecane and dodecanol.

The results for the two *bicolor* species from Tunisia show them to be similar to *C. niger* and *C. nodus*, but there are differences evident. *Cataglyphis bicolor*-1 has more tridecane and dodecanol than the others and less petandecanone than the Syrian species. *Cataglyphis bicolor*-2 has much less of the oxygenated compounds than the others in this group. *Cataglyphis mauritanicus* is not particularly close to *C. altisquamis*, the other species from the same group, but the differences between the individual nests of *C. mauritanicus* makes conclusions difficult.

The correlation with results obtained by Hefetz and Orion (1982) for two out of three of apparently the same species is not good. Firstly, Hefetz and Orion (1982) record only hydrocarbons and no oxygenated compounds. Secondly, they found much larger differences between *C. nodus* and *C. niger.* Their results for *C. nodus* were roughly similar to our own (pentadecane 68%, tridecane 15%) but quite different for *C. niger* (tridecane 59%, undecane 25%, pentadecane 12%). Very unusually, they found *C. isis* to have an even-numbered hydrocarbon as main component (hexadecane 58%, tridecane 30%). Possibly dodecanol was mistakenly identified as hexadecane. Otherwise one must conclude that *C. isis* from Israel has a different composition or there is confusion in identifying some of these species.

Mandibular glands

The preliminary examination of glandular material showed the predominance of two compounds in mandibular glands, citronellol and a low molecular mass primary alcohol, but a number of the mandibular glands were empty. It was thought doubtful if mandibular glands would be a useful marker. In the larger sample from Syria it was clear that in every case except *C. ruber* mandibular gland contents were a useful marker of species, as illustrated by Table 7. Although the mandibular secretions of *C. altisquamis* and *C. frigidus* are indistinguishable, the species are easily differentiated by their Dufour gland secretions. The five species that contain chiefly 2-methyl-1-hexanol (*isis, niger, nodus, bicolor*-1 and *bicolor*-2) appear on this basis to be a distinct subgroup from those that contain chiefly citronellol. *C. bicolor*-1 appears to be distinguished from *bicolor*-2 by the larger proportion of citronellol in the former, but because of the larger variations between samples this could only be confirmed by larger samples. *Cataglyphis mauritanicus*, by its mandibular secretion fits a group with *altisquamis*, but would fit equally well with *frigidus*, where there is more resemblance between the Dufour gland secretions. Our results differ from those of Hefetz and Lloyd (1985) who found citronellol in *C. niger* and also in *C. bombycinus*, where it was accompanied by geraniol. We found no volatile substances in the mandibular glands of *C. savignyi* (Ali *et al.*, 1988), but the variability whereby some glands were empty and some not, and even one mandibular gland from the same forager contained material and the other did not, suggests that these glands may be discharged during capture of the ants or during dissection. It is nevertheless puzzling how the glands can be so completely discharged that not even one-fiftieth part of the secretion is detectable.

2-Methyl-1-hexanol represents an entirely new mandibular gland substance as well as being found as a natural product for the first time. The closest parallel is the finding of 2-methyl-1-butanol in the ventral abdominal gland of dictyopteran adults (Waterhouse and Wallbank, 1967).

The total amount in the glands of many species are found to vary around quite large limits. The mean amounts (in ng) found in the Dufour and mandibular glands of the seven species from Syria are shown graphically in Fig. 2. They were all rather similar except *C. altisquamis* which has noticeably larger glands. In every case, the Dufour glands contain more material than the mandibular glands.

All the Syrian species are easily distinguished by the combination of substances in

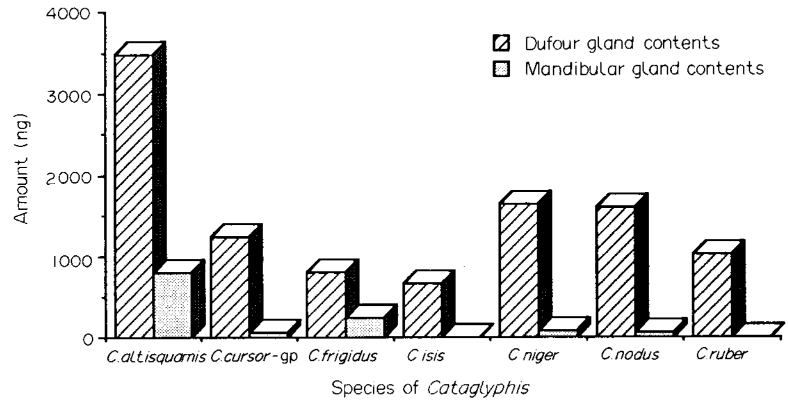

FIG. 2. THE RELATIVE AMOUNTS OF MATERIAL FOUND IN THE DUFOUR AND MANDIBULAR GLANDS OF THE SEVEN SPECIES OF *CATAGLYPHIS* COLLECTED IN SYRIA.

their mandibular and Dufour glands, with the possible exception of the two *bicolor* group species *C. niger* and *C. nodus.* Although their mean percentage compositions are different, because of the natural variations between individuals and colonies, it is conceivable that samples could be collected where it would not be possible to decide on chemical grounds to which species it belonged. Fortunately, these two are easily distinguished by the major compound of their postpharyngeal gland, as mentioned earlier. This is pentacosane in *C. nodus* and a mixture of 11-methylnonacosane and 13-methylnonacosane, not separated by our chromatography column, in *C. niger.* This is the first recorded example of species being diagnosed by the contents of the postpharyngeal gland. However, this gland contains complex mixtures of hydrocarbons, and at present too little is known about it to use it generally as a species-recognition factor.

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